

B1
contd

below) and used as the "capture" oligonucleotide. It is also used as the soluble inhibitor in competition assays. A second oligonucleotide sequence, complementary in sequence to the T3 promoter, is synthesized at the same service facility, fluorescently-labeled and used as the soluble "tracer" oligonucleotide in DNA hybridization assays. To this end, the oligonucleotide is synthesized with a terminal amino group on the 3' end and labeled with Cy-5 (Molecular Detection Systems, Pittsburgh), a red-emitting fluorescent dye.

Please replace the second full paragraph on page 28 that carries over to page 29 with the following:

B2

Assay I is a direct binding assay between two complementary oligonucleotides. The T3 RNA polymerase promoter site was chosen as a model system for our feasibility studies. The T3 promoter is a region spanning 20 bases with the following sequence: 5' AATTAACCCTCACTAAAGGG 3' (SEQ. ID. NO. 1). An oligonucleotide (T3 promoter) with this sequence was synthesized, biotinylated at the 5' end (via a spacer) and immobilized to waveguides (silica or polystyrene) coated with either avidin or streptavidin. A second nucleotide with a complementary sequence (anti T3) was also synthesized and labeled with Cy-5, a red-emitting fluorescent dye. Solutions with increasing tracer concentration were exposed to the waveguide and the fluorescence of bound tracer was plotted versus soluble tracer concentration. Assay II is a competitive binding assay in which unlabeled Anti T3 competes with Cy-5 labeled Anti T3 for binding to immobilized T3 promoter. In this case different concentrations of unlabeled Anti T3 were mixed with a fixed concentration of labeled Anti T3 (tracer). The fluorescence of bound tracer was measured for each mixture and plotted versus unlabeled Anti T3 concentration.

Please replace the footnote under Table 2 on page 29 with the following:

B3

†Abbreviations: Cy-5, an amino-reactive red-emitting fluorescent dye produced by Biological Detection Systems (Pittsburgh, PA); T3 Promoter, the transcriptional promoter site of T3 RNA polymerase which consists of the sequence 5' AATTAACCCTCACTAAAGGG 3' (SEQ. ID. NO. 1); Anti T3, oligonucleotide with a complementary sequence to the T3 promoter.
